REMARKS

Drawings:

A Petition under 3 CFR 1.84(a)(2) to permit color drawings was filed concurrently with the application on September 4, 2003, a copy of which is attached for the Examiner's convenience. The specification as filed contains the required language for color drawings, see page 9.

Claim Objections:

The specification has been amended to address the informalities noted on page 2 of the Office Action.

Claim Rejections Under 35 USC 112, First Paragraph:

Claims 1-10 were rejected as failing to comply with the enablement requirement of 35 USC 112, second paragraph. Essentially, the Examiner asserts that extension of DNA would be "impossible" where the extension temperature is greater than the primer Td, reasoning that the primer set should become functionally dissociated from the template in such a situation. However, the successful use of higher extension temperatures relative to primer Td in the practice of the invention, and indeed, in standard automated PCR-based cycle sequencing reactions, demonstrates that extension does in fact occur under these conditions. The fundamental reason is that primer annealing in the presence of a polymerase and nucleotides results in substantial primer extension within a matter of seconds. Therefore, if the "annealing" temperature is maintained even for a few seconds, primer extension will already begin to occur. The ~30 second annealing step of the methods of claims 1-9, for example, is far longer than the time required to initiate DNA synthesis, and is optimal for producing excellent read-lengths

through difficult GC-rich sequencing targets, as has clearly been established by the specification. Raising the temperature thereafter, in order to provide the polymerase with better reaction kinetics and extend the read-length as much as possible, simply does not prevent or terminate continuing extension.

In any type of PCR-based cycle sequencing reaction, primers are used to initiate second strand synthesis from a single-stranded template DNA. Basically, the free OH group at the 3' end of a primer enables the polymerase present in the reaction mixture to *immediately* commence extension of the primed second strand in the presence of deoxy and dideoxy nucleotides. Once annealing of the primer to the template has taken place, the polymerase will initiate the addition of nucleotides, and in only a second will extend the primer sufficiently to raise the Td of the second strand being created. Thus, the Td of the primer rises and continues to rise as the primer is extended. In only a few seconds, primers are annealed to template DNA and the polymerase is off and running down the template and extending the second strand, which now has a Td that can withstand the higher temperatures subsequently used for optimal polymerase extension. In other words, the dynamics change rapidly.

Therefore, the question of primer dissociation from the template strand to which it is annealed is not a question of pure hybridization or dissociation, because the composition of the primer is changed by the extension reaction occurring during the annealing step. Additionally, whether or not the primer portion of the second strand becomes functionally dissociated by the higher temperatures used during the "extension" cycle of the process obviously does not affect the polymerization of the growing second strand, as the successful use of higher extension temperatures relative to primer Td in standard cycle sequencing reactions plainly establishes. In addition to the generation of a double stranded DNA that requires higher and higher temperatures for strand dissociation, the polymerase rapidly moves far downstream of the primer initiation site, where it continues to

polymerize, within the annealing step timeframe. In summary, the Td of the primer, *per se*, is no longer a critical factor.

It is important to recognize that in any such reaction, there are many hundreds of thousands of molecules being primed and extended. As is well known in the art, successful PCR-based cycle sequencing reactions are predicated on the idea that a sufficient number of desired molecular interactions will take place under the conditions used, not on the idea that each and every molecular interaction will take place in precisely that way.

With respect to the claimed invention, annealing is conducted at higher temperatures, compared to standard sequencing reaction conditions, in order to maintain an open DNA conformation in situations where the nature of the template DNA (i.e., high GC content) can result in secondary structures in the template DNA (i.e., hairpins) which prevent the polymerase from "reading-through" the template strand. The high Td primers used in the practice of the invention anneal quite well at such higher temperatures, *by design*. As explained above, once the primers are annealed to the single stranded template and second-strand DNA synthesis commences, raising the temperature for the "extension" cycle does not terminate extension, as the polymerase present in the reaction mixture has already been "activated" by the annealed primers and the dynamics have rapidly changed. The invention utilizes higher temperatures for extension in order to maximize the opportunity to keep confounding secondary structures from forming and maintain the linear state of the template DNA, thereby providing the polymerase with a "open road" for processing through as much of the template as possible.

The methods of the invention clearly work. In addition to the working examples presented in the specification, the inventor established the claimed ranges for primer Td, annealing, extension, etc., through numerous successful (and unsuccessful) sequencing reactions. The results and knowledge gained from the

inventor's extensive empirical observations are disclosed in the specification. See, for example, the disclosure of successful annealing temperature conditions on page 23, wherein the specified ranges are stated to have resulted from testing various conditions. Example 2 provides evidence of success within the range of conditions that were established by the inventor's experimental activities.

The Examiner states that the invention is broadly claimed, and that there is a lack of working examples present in the specification. This reasoning appears to be mainly based on a misperception of "uncertainty" regarding the use of extension temperatures in excess of primer Td. Applicants have addressed this misperception, above, and request reconsideration of the breadth of the claims in view of the explanations provided herein. Applicants contend that the claims are precisely drawn to cover the particular condition ranges that empirical evidence established, and are certainly not overly broad so as to require a plurality of working examples. Example 2 provides a working example of the methods of claims 1-9, while Example 3 provides a working example of the method of claim 10, which is drawn to a sequencing method distinct from the one claimed in claims 1-9 (the Examiner incorrectly states that *"the extension of Example 3 is not within the claimed range* [of claims 1-9]). Example 3 relates to claim 10, not claims 1-9. In the case of Example 2, results are provided for three different GC-rich templates. Thus, *all* claims are well supported by specific disclosure based on empirical evidence and by working examples, and are therefore fully enabled by the specification.

In view of the foregoing, applicants respectfully request reconsideration and withdrawal of the rejections of claims 1-10 for lack of enablement.

Claim Rejections Under 35 USC 112, Second Paragraph:

Claims 1-9 were rejected as being indefinite in several respects. Claim 1 has been amended to replace the term *"at least about"* in steps (b) and (c) with *"about"*. Claim 1 has been further amended to insert *"sample"* before *"DNA"* in step (b) for clarity.

However, applicants respectfully disagree with the Examiner's characterization of the terms *"the reaction mixture"* in step (e) and *"the series"* in step (g) as lacking antecedent basis. The term *"the reaction mixture"* in step (e) finds antecedent basis in step (a), *"preparing a reaction mixture"*. The term *"the series"* in step (g) finds antecedent basis in step (d), *"extending the annealed primers to generate a series of fluorescently-labeled dideoxynucleic acid fragments"*.

Conclusion:

In view of the foregoing amendments to the claims and remarks, applicants respectfully request withdrawal of all pending rejections and an indication of allowability of claims 1-10. If in any respect the Examiner is not persuaded, and/or has questions about the claimed invention or the underlying technology, the undersigned would greatly appreciate an opportunity for a telephone conversation, with or without the inventor as necessary.

Respectfully submitted,

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Signature of Attorney

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